1972

Secretory Vesicle Formation and Dictyosome Morphology in Tetrasporangia of the Marine Red Alga Polysiphonia denudata (Dillwyn) Kutzing

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SECRETORY VESICLE FORMATION AND DICTYOSOME MORPHOLOGY IN TETRASPORANGIA OF THE MARINE RED ALGA POLYSIPHONIA DENUDATA (DILLWYN) KUTZING

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

Charles Dickson Alley, Jr.

1972

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

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ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr. Joseph L. Scott, under whose guidence this investigation was conducted, for his patient guidance and criticism throughout the investigation. The author is also indebted to Dr. Robert E. L. Black and Dr. Lawrence Wiseman for their careful reading and criticism of the manuscript.

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ABSTRACT

The production of dictyosome-derived vesicles was investigated at the ultrastructureal level in mature or stage III tetrasporangia of the marine red alga Polysiphonia denudata. Stage III is the post-meiotic stage of the tetrasporangium during which cytokinesis is in progress or already completed and where a mucopolysaccharide layer continuous with the cleavage furrows is produced. It is apparent that the secretory product of hypertrophied dictyosomes contributes to this mucopolysaccharide layer. The dictyosomes are characterized by a minimal intercisternal spacing which is maintained by a conspicuous osmiophilic cementing substance. Electron opaque material is observed in the cisterna at the proximal pole of the dictyosome while electron transparent material progressively accummulates toward the distal pole. Vesicles are produced by the centripetal inflation of the distal cisterna and only a single vesicle per cisterna appears to be formed. When the distal cisterna becomes fully inflated, electron transparent material completely surrounds the opaque substance creating the dark-cored appearance characteristic of the derived vesicles found during this stage of tetrasporogenesis. To my knowledge, the intercisternal cementing substance which is responsible for keeping the dictyosome together as a discrete organelle has not been observed in other plant or animal cell types. Also, hypertrophied dictyosomes producing only a single vesicle from each cisterna and the apparent mechanism by which this is accomplished have not been previously reported.

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INTRODUCTION

The Golgi apparatus in most plant cell types is reported to be composed of dictyosomes or polarized stacks of cisternae. Dictyosomes are found situated so that their proximal poles or forming faces are adjacent to either endoplasmic reticulum or the nuclear envelope. Several studies provide evidence that the cisternae at the proximal pole of the dictyosome are formed from the fusion of small vesicles or blebs believed to originate from the endoplasmic reticulum or the outer membrane of the nuclear envelope (14). The continued production of new cisternae at the proximal pole results in the displacement of each of the previously formed cisternae from the proximal toward the distal pole. As each new cisterna is formed at the proximal pole one is apparently lost through vesicle production at the distal pole, since the number of cisternae appear to remain constant in a given dictyosome (14).

In studies on the hyphae of the fungus Pythium ultimum it was observed that during the cisternal progression from the proximal pole to the distal pole the cisternal membrane characteristics changed from those of the endoplasmic reticulum to the type exhibited by the plasma membrane (7,8). In dictyosomes of other organisms the intercisternal staining characteristics also appear to change during this membrane transformation, resulting in the appearance of a material with staining characteristics similar to that of the contents of the

secretory vesicles produced at the distal pole of the dictyosome (4,8, 19,26).

In addition to membrane transformation and product staining characteristics, the development of secretory vesicles is observed to be progressive across the dictyosome. This development begins with the accummulation of the products of synthesis within the cisternae. As the cisternae advance to the distal pole, they become progressively inflated and filled with additional product. Light and electron microscopic cytochemistry (3,19,21,26), autoradiography (18) and biochemical analysis of isolated dictyosomes (23) show a localization of polysaccharide and other complex carbohydrates in the Golgi apparatus and its derived vesicles. Whalely suggests that the Golgi apparatus contains the enzymes necessary for the synthesis of certain carbohydrates (25) and others give evidence indicating that the Golgi cisternae and vesicles are the site of the complexing of carbohydrates with proteins synthesized in the endoplasmic reticulum (3).

Although the foregoing is reasonably well documented, little is known of the fate of the dictyosome cisternae in plants following the release of secretory vesicles. Mollenhauer (11) postulated that in maize the cisternae dissociate from the distal pole of the dictyosomes following vesicle release. The dissociated cisternae fragment and are quickly broken down beyond the resolution of the electron microscope.

The major concern of this study is directed toward the role of the cisternae in vesicle production in tetrasporangia of the marine red alga Polysiphonia denudata. Observations of dictyosomes in these cells are presented showing a previously unreported method of vesicle

production which directly takes into account the majority of the membrane of the distal-most cisterna.

MATERIALS AND METHODS

Tetrasporophytes of Polysiphonia denudata (Rhodophyta, Rhodomelaceae) were collected from the York River near Yorktown, Virginia, during the summer months of 1971. The material was gathered during both high and low tides and was found attached to rocks and shells at shallow depths.

Most thalli were fixed immediately following collection in a mixture of 3% paraformaldehyde - 3% glutaraldehyde in a 0.1 M phosphate buffer (pH 6.6) with 0.15 M sucrose, while others were stored in sea water for later observation with the light microscope. Post fixation was performed using 1% OsO_4 in the same buffer. Both fixations were over a two hour period. Early during the dehydration schedule using increasing concentrations of acetone, the thalli were stained 16-24 hours in a 2% uranyl acetate - 70% acetone solution at 4^{o} C. Epon 812 was used to infiltrate and embed the thalli (9).

Thin sectioning was done with a diamond knife on a Porter-Blum MT-2B ultramicrotome. The sections were stained briefly in lead citrate (24) before examination in a Zeiss EM 9S-2 electron microscope.

Epon-embedded material prepared for electron microrcopy was cut with a glass knife at 1.5-2.0 u and was stained with toluidine blue or the PAS reaction. All material examined by light microscopy, both living and resin embedded, was photographed with a Zeiss Photomicroscope II on 35 mm Panatomic X film.

RESULTS

The marine red alga Polysiphonia denudata exhibits a typical Polysiphonia type life history. In short, this entails an alternation of morphologically identical macroscopic gametophyte and tetrasporophyte generations (5). All observations were made on early, mid and late stage III tetraspores of the tetrasporophyte plant. Stage III is the post-meiotic stage of the tetrasporangium in which cytokinesis is either in progress or already completed (20) (Figs. 2 and 3). At this stage in the development of tetraspores the dictyosomes are in a hypertrophied condition.

Dictyosomes in the mature tetrasporangia are characterized by highly inflated cisternae associated with large vesicles. The vesicles are characteristically electron transparent with moderately dense fibrous cores and are located near the distal pole (Fig. 4). The approximate length of cisternae in hypertrophied dictyosomes is 3-4um while in comparison, the cisternae of the vegetative dictyosome in P. denudata measures about o.5-0.6um in length (Fig. 5).

The dictyosomes appear to have no specific orientation with respect to either the nuclei or the investing wall layers. Also there is no specific orientation of the distal pole either toward or away from the large fibrous vacuoles that are observed in stage III tetraspores. However, these vacuoles are found only in areas of the cytoplasm where several dictyosomes are present. Although dictyosomes

appear to be randomly located in the cytoplasm there is one organelle that appears to be specifically associated with the dictyosomes. A single mitochondrion is always observed to be associated with the proximal pole of a dictyosome (Figs. 5 and 7).

Smooth endoplasmic reticulum is also observed at the proximal pole of the dictyosomes. Small vesicular bodies appear to be formed and released by the endoplasmic reticulum (Figs. 6 and 7). As the "blebs" are released by the endoplasmic reticulum, they become either coated or filled with an osmiophilic substance. Observations give evidence that these electron dense bodies coalesce with the newly forming cisternae (Fig. 7).

Beginning with the second and third cisternae from the proximal pole of the dictyosome, the central portion of the adjacent cisternae are appressed (Fig. 6). The intercisternal area in this region stains intensely giving the appearance of membrane fusion. Under higher magnification the appearance of the region seems to be due to the presence of an intercisternal substance and not to direct membrane fusion (Fig. 8). Further evidence for an intercisternal substance is obtained through measurement of the membranes of the cisternae. A single cisternal membrane is approximately 5.5-6.5nm wide, whereas the width of the electron dense region where the two membranes are in close apposition is 15-17nm. This distance is significantly larger than that expected if the membranes were actually fused.

The product of the dictyosome is first noted in the lumen of the proximal cisternae as a moderately electron dense, dispersed substance (Fig. 6). With the appearance of the above mentioned intercisternal

substance, another substance is seen to be aggregating within the lumina at the periphery of the cisternae. The accummulation of the electron transparent material is evidenced by the appearance of cisternal inflation. Possibly due to a continued production of this material, an expansion of the cisterna progresses centripetally (Fig. 7). As the cisterna continues to expand the moderately electron dense substance first noted appears to be condensed in the central portion.

The intensely staining intercisternal substance disappears when a cisterna reached a position six to eight cisternae from the proximal pole of the dictyosome (Figs. 7 and 8). At this location the expansion of a cisterna is rather extensive. The central portion, although still containing only the moderately staining material, has expanded to approximately twice its original size (Fig. 7). The entire nascent secretory vesicle now become completely separated from the stack and continues to expand (Figs. 6 and 7). The cisternal expansion persists in the same manner as prior to its release from the dictyosome. The electron transparent material finally surrounds the darker substance producing a single vesicle from the entire cisterna. These vesicles appear to contribute to the large fibrous vacuoles prevalent in these cells (Figs. 4 and 9).

Light microscopic cytochemical techniques using both toluidine blue and the PAS reaction show the contents of the large fibrous vacuoles in P. denudata to be carbohydrate in nature (Fig. 2). Peyriere, in recent histochemical and cytochemical studies on the dictyosomes of Griffithsia flosculosa has determined the material contained in the

secretory vesicles to be mucopolysaccharide (17). Therefore it is probable that the product of the dictyosomes is a complex polysaccharide.

DISCUSSION

Observations on the tetraspores of the marine red alga Polysiphonia denudata show a consistent relationship between a single mitochondrion and the proximal pole of the dictyosome. As early as 1956 Lever (in 2) postulated the existence of such a relationship. Recent work on the ultrastructure of the Rhodophyta show that this may indeed be a diagnostic character of this division of the algae (20). Other workers have also reported this relationship in several other organisms (1,12,16,17). Mitochondria are known, in some cells, to be located in close proximity to other organelles that require ATP to carry out their normal function (i.e., cardiac tissue) (6). The work of Schnepf with the use of inhibitors, supports the hypothesis that there is a dependence of the individual dictyosomes upon respiratory energy (in 12). Therefore, the observation of a mitochondrial-dictyosome relationship could very well be explained on the basis of a primitive energy supply - energy usage relationship.

The formation of the cisterna at the proximal pole of the dictyosome is well documented in several organisms (14). However, in P. denudata tetraspores the vesicles that are released from the endoplasmic reticulum are coated with an osmiophilic substance. Since this darkly staining substance does not appear on the outer faces of the membranes of the endoplasmic reticulum or when the vesicles coalesce to form the first cisterna, I can only conjecture as to its fate or function.

Three possible functions are: (a) that this material is a portion of the secretory product that is synthesized by the endoplasmic reticulum and is eventually incorporated within the lumen of the cisterna; (b) that it is a material effecting the changes in membrane structure as reported by Bracker (7); or (c) that this material is the intercisternal bonding component.

The morphology of dictyosomes in P. denudata essentially follows the classical description which has attained wide support (14). The two major deviations from the classical model are the appearance of an osmiophilic intercisternal cementing substance and the method of vesicle formation.

The existence of an intercisternal substance in these dictyosomes became apparent after measurement of cisternae and close examination of the staining characteristics of the area where membrane fusion was thought to be exhibited. The measurements given earlier indicate that the distance from the membrane of one cisterna to the contiguous membrane of the next cisterna in the 'fused' regions was greater than the sum of the width of two cisternal membranes in the 'non-fused' region. Also the electron opaque outer layers with a faint inner fused layer characteristic of fused membranes, i.e., the so-called tight junctions, were not observed (15) (Fig. 8). Mollenhauer and Morre gave indirect evidence for some type of intercisternal bonding material which functions to hold the cisternae of the dictyosome together (12). Their evidence consisted of the following: (a) that dictyosomes could be isolated as intact individual organelles; (b) that their characteristic morphology was not disrupted by stratification in the intact cell; and (c) that

there existed a constant intercisternal spacing both in the intact cells and when isolated. This last piece of evidence is of special interest her since the present work shows that as one proceeds to the distal pole there is a decrease in the amount of the cisternae in which this minimal intercisternal distance is maintained. The intercisternal substance is observed to be confined to these minimal distance areas, and therefore, also decreases in amount from the proximal to the distal pole (Fig. 7). In contrast to previous reports on discrete, tubular intercisternal elements (10,22), in P. denudata this material appears instead to fill the entire intercisternal space. Although the intercisternal elements reported in the dictyosomes in other organisms are not believed to be the cemmenting substance (13), in this alga the intercisternal material appears to be the cementing substance and constitutes the first tangible evidence for this substance in either plant or animal cells.

In P. denudata tetraspores a single vesicle is formed from each cisterna at the distal pole of the dictyosome. The entire distal cisterna appears to be involved in the production of the vesicle, rather than exhibiting the peripheral secretory and central non-secretory portions of the classical model (14). Beams and Kessel, in their review article, briefly alluded to a similar pathway of vesicle formation shown in a single micrograph (2).

The process of vesicle formation in the dictyosomes of P. denudata is apparently due to the manner of product accummulation. Therefore, vesicle development begins with the appearance of the intercisternal material, which marks the beginning of product accummulation (Fig. 7). The periphery of the cisternae inflate first probably due to lateral

product accummulation. As the product continues to be modified the cisternal expansion progresses centripetally. Wherever inflation of the appressed portion of the cisternae has begun the intercisternal cementing substance is not observed and the distance between the peripheral regions of the opposed cisternae increases. With the expansion of the central portion and the complete disappearance of the cementing substance the cisterna (nascent secretory vesicle) is released from the dictyosome (Fig. 7). This release appears to be complete since those cisternae that are spatially separated from the cisternal stack often lose their previous orientation with regard to the stack (Figs.6 and 7). The released cisternae continue to expand and eventually become the spherical secretory vesicles.

Further evidence that vesicle formation is directed from the extremes toward the center is derived from the stainingcharacteristics within the cisternae as they progress from endoplasmic reticulum to secretory vesicle. Due to their differential electron transmission one observes that there are at least two different intracisternal materials at this stage of tetraspore development. The first to appear is the moderately staining material which is confined to the central portion of the cisternae of the stack and the cisternal bridge connecting the vesicular lobes of the released nascent secretory vesicle.

This material also appears as the dark core of the vesicles that are formed. This implies that the electron transparent substance that is observed at the ends of the cisternae confine this first electron dense material to the central portion of the cisternae, finally surrounding it completely after the cisternae have been released from the dictyosome

stack.

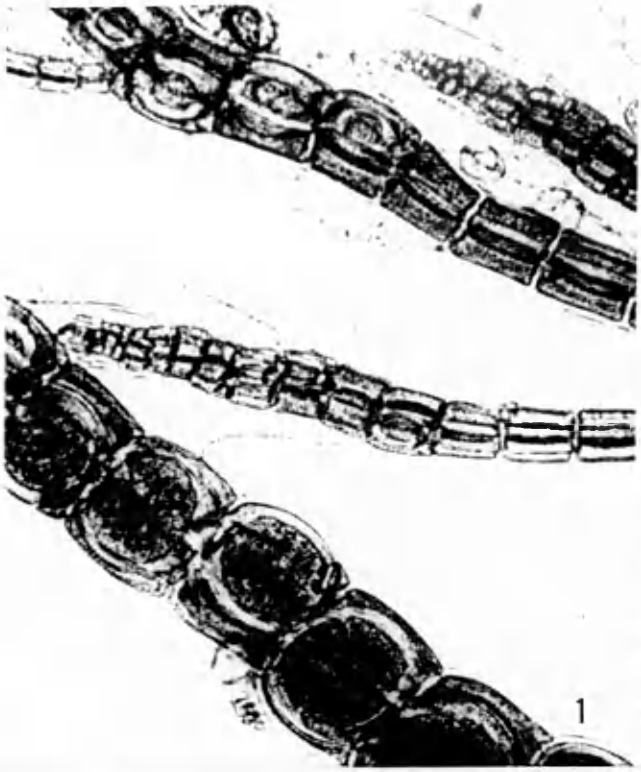
The red algae have long been considered to be one of the most primitive groups of plants, and therefore the apparent lack of consistency of these observations with classical models based on more highly evolved cell types should not be of any great surprise. At the present time the cementing substance responsible for keeping the dictyosome together as a discrete organelle has not been observed in any other cell type, plant or animal. Also, hypertrophied dictyosomes which produce but a single vesicle from each cisterna and the apparent mechanism by which this is accomplished, have not been previously reported. These unique observations in the tetraspores of P. denudata may possibly be characteristic of a primitive cell type that evolved separately from those types which were the predecessors of the cells of more advanced plants and animals.

PLATE 1

Figure 1. Living thalli of Polysiphonia denudata exhibiting internally borne tetrasporangia in several stages of development. Normarski differential interference contrast, X224.

Figure 2. Light micrograph of a 1 u thick section through a thallus containing nearly mature tetrasporangia. Toluidine blue, X560.

Figure 3. Low magnification electron micrograph of a tetrasporangium in which cytokinesis is occuring (stage III). N, nucleus; CF, cleavage furrow; FV, fibrous vacuole; X1,960.



1

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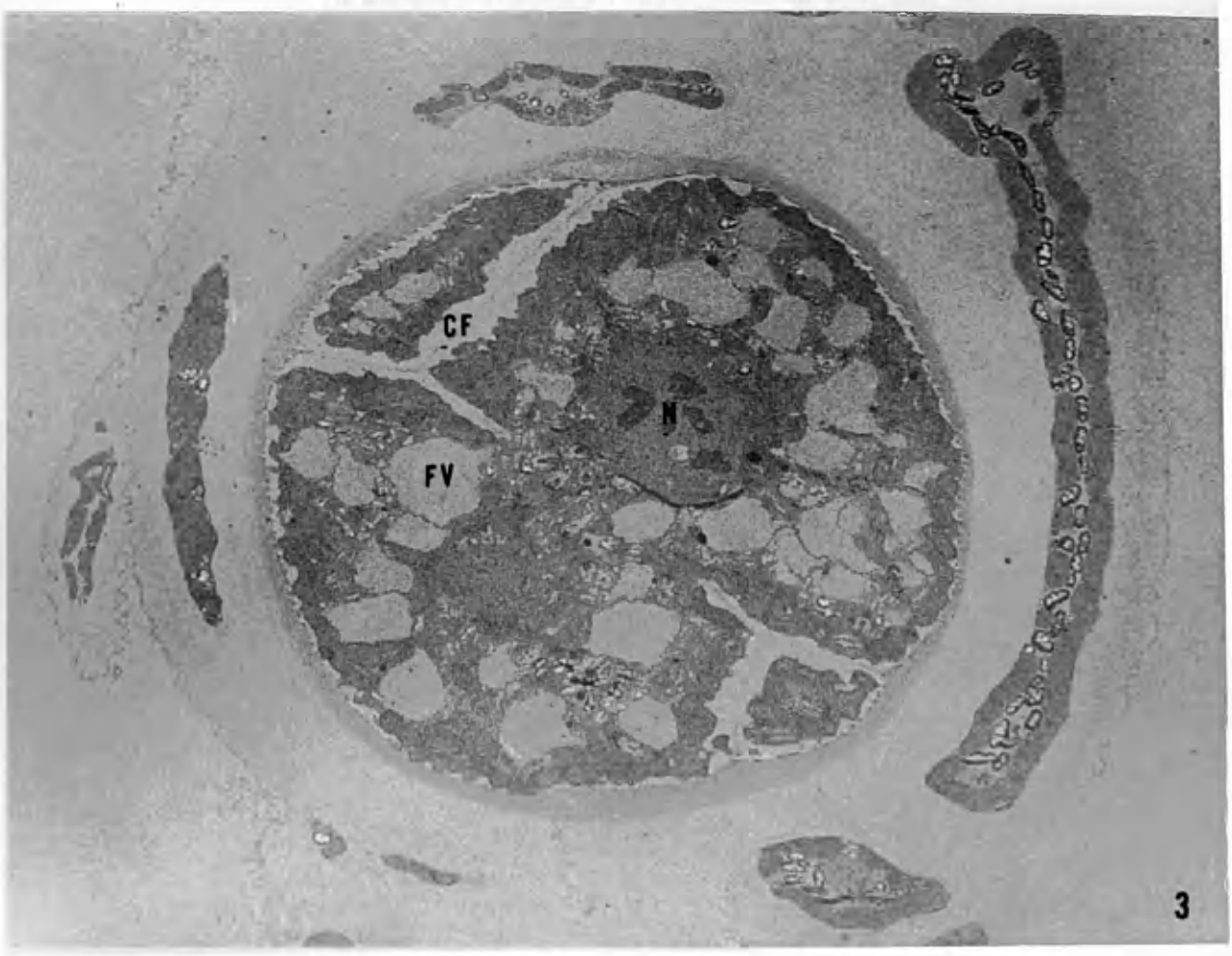

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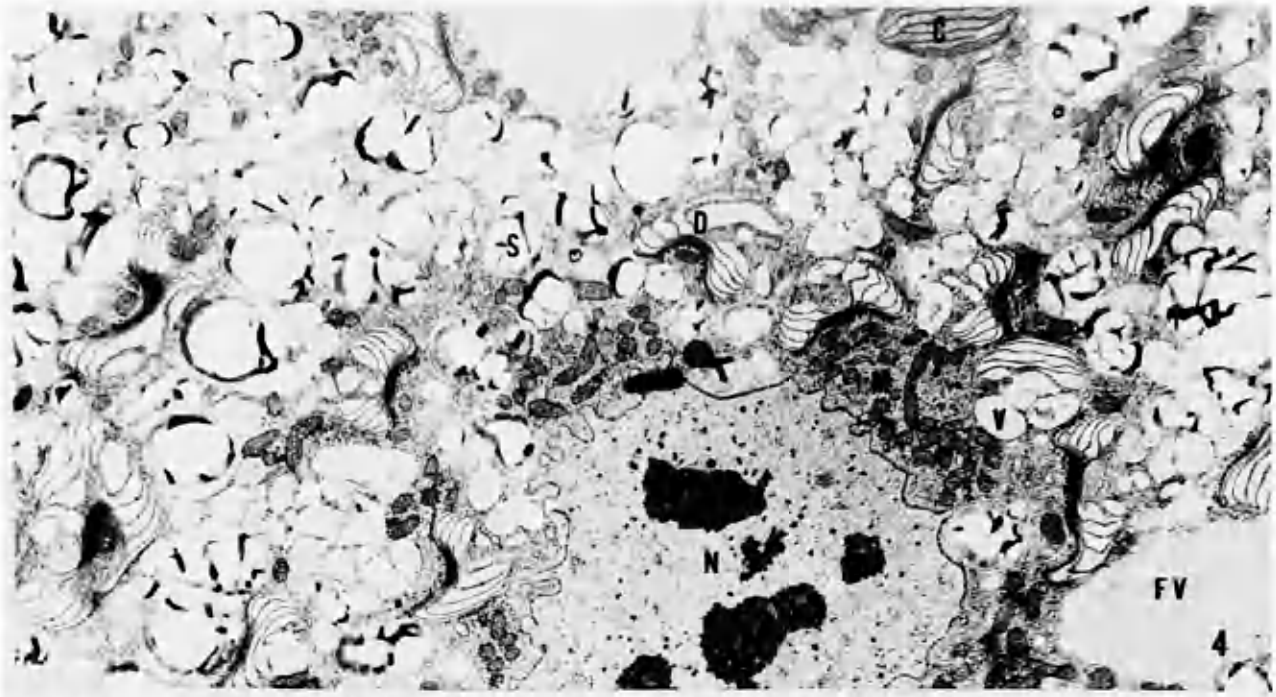
PLATE II

Figure 4. Electron micrograph showing dictyosomes (D) in the hypertrophied state apparently producing the large vesicles (V). The dictyosomes appear to have no specific orientation with respect to the other subcellular organelles. C, chloroplast; N, nucleus; FV, fibrous vacuole; M, mitochondrion; S, starch grain. X42,750.

Figure 5. Dictyosomes (D) of a vegetative cell with associated mitochondria (M). Note the lack of cisternal inflation and vesiculation at the distal pole. C, chloroplast. X23,750.

Figure 6. Electron micrograph exhibiting the apparent formation of blebs (arrow) by the endoplasmic reticulum (ER) and the accummulation of these blebs along the proximal pole of the hypertrophied dictyosome. An intercisternal sunstance (double arrow) is observed in the appressed portion of the dictyosome cisternae. M, mitochondrion; NSV, nascent secretory vesicle. X49,875.


C
S
D
V
N
FV
4


M
5
M
C
D
D

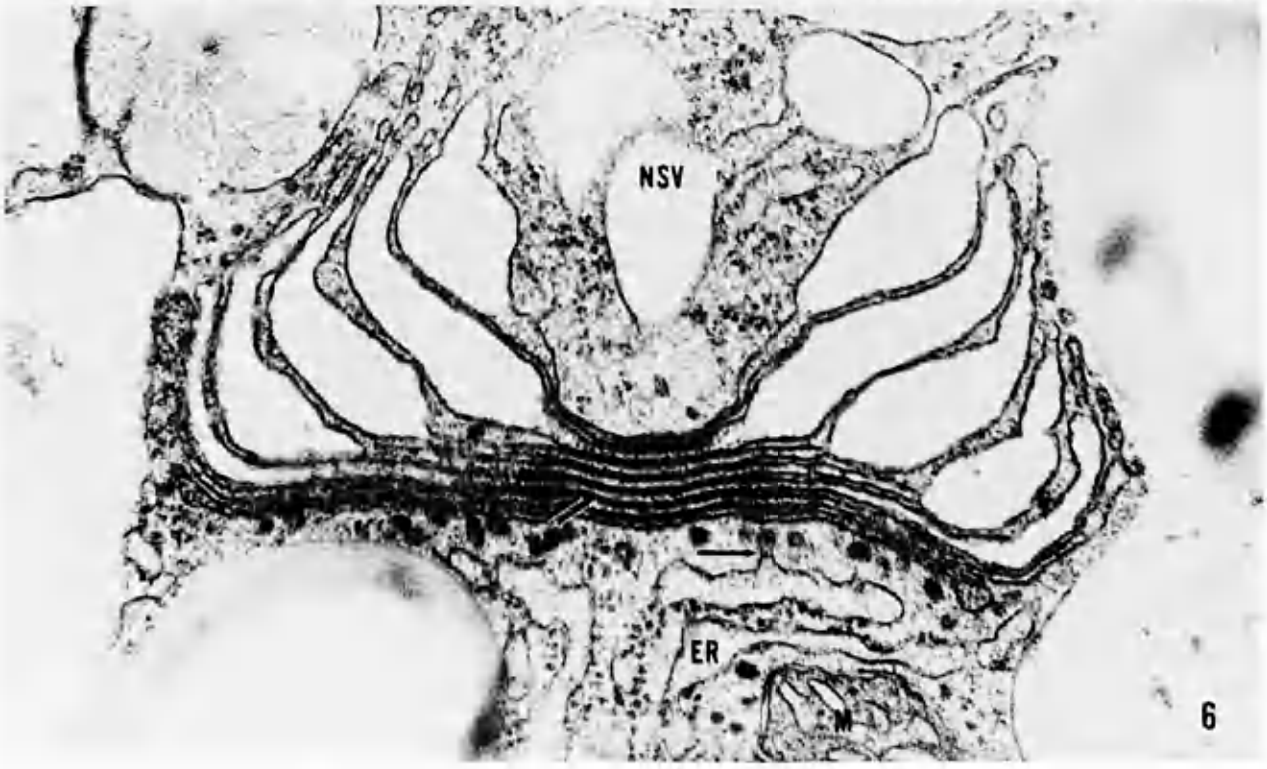

NSV
ER
M
6

PLATE III

Figure 7. Section through a dictyosome in the hypertrophied state giving evidence of the formation of secretory vesicles by the separation and centripetal expansion of the distal cisterna. Also, note the apparent formation of the proximal cisterna from the coalescence of blebs from the endoplasmic reticulum (ER) (arrow). DC, distal cisterna; NSV, nascent secretory vesicle; V, secretory vesicle; M, mitochondrion. X69,225.

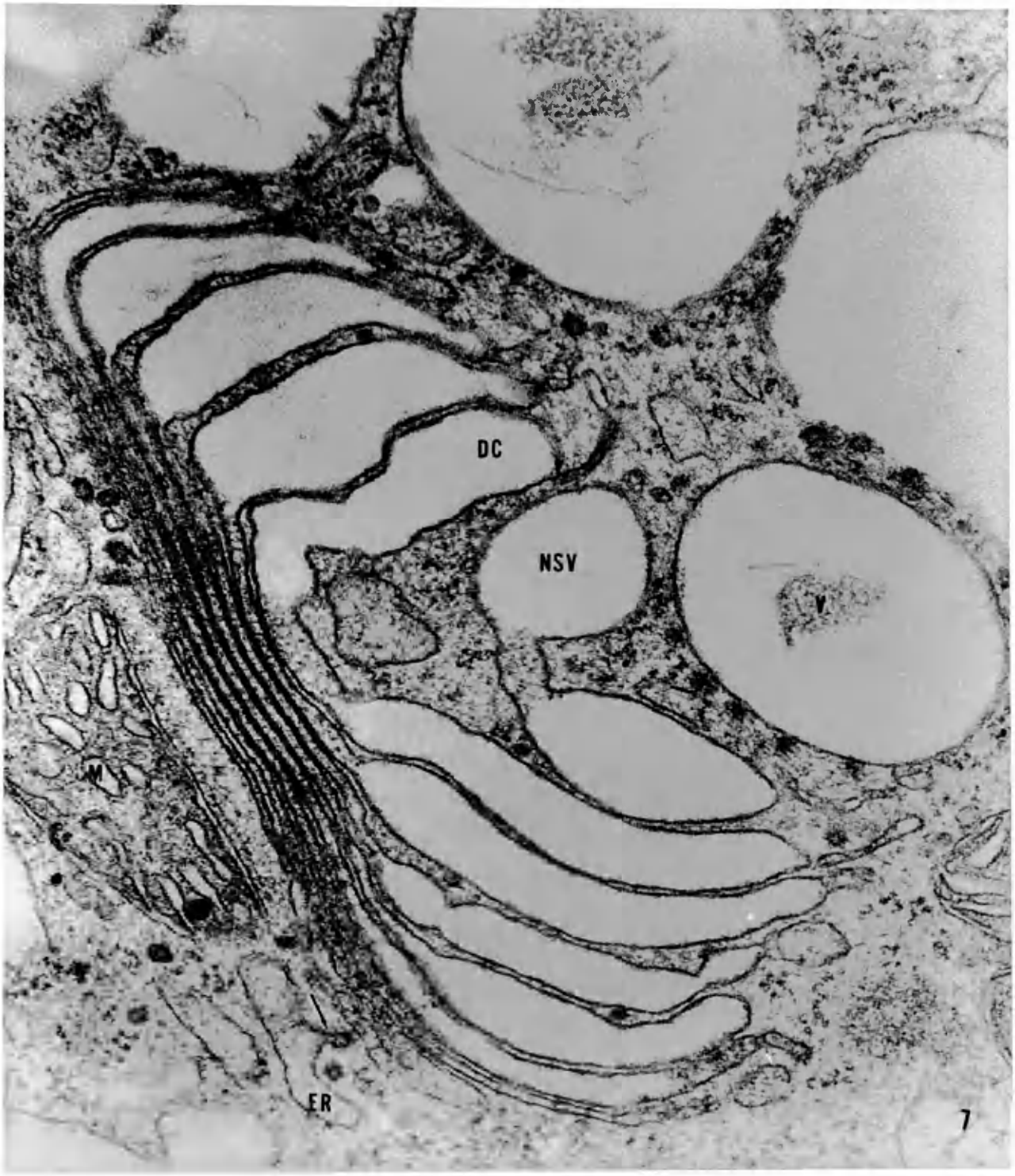
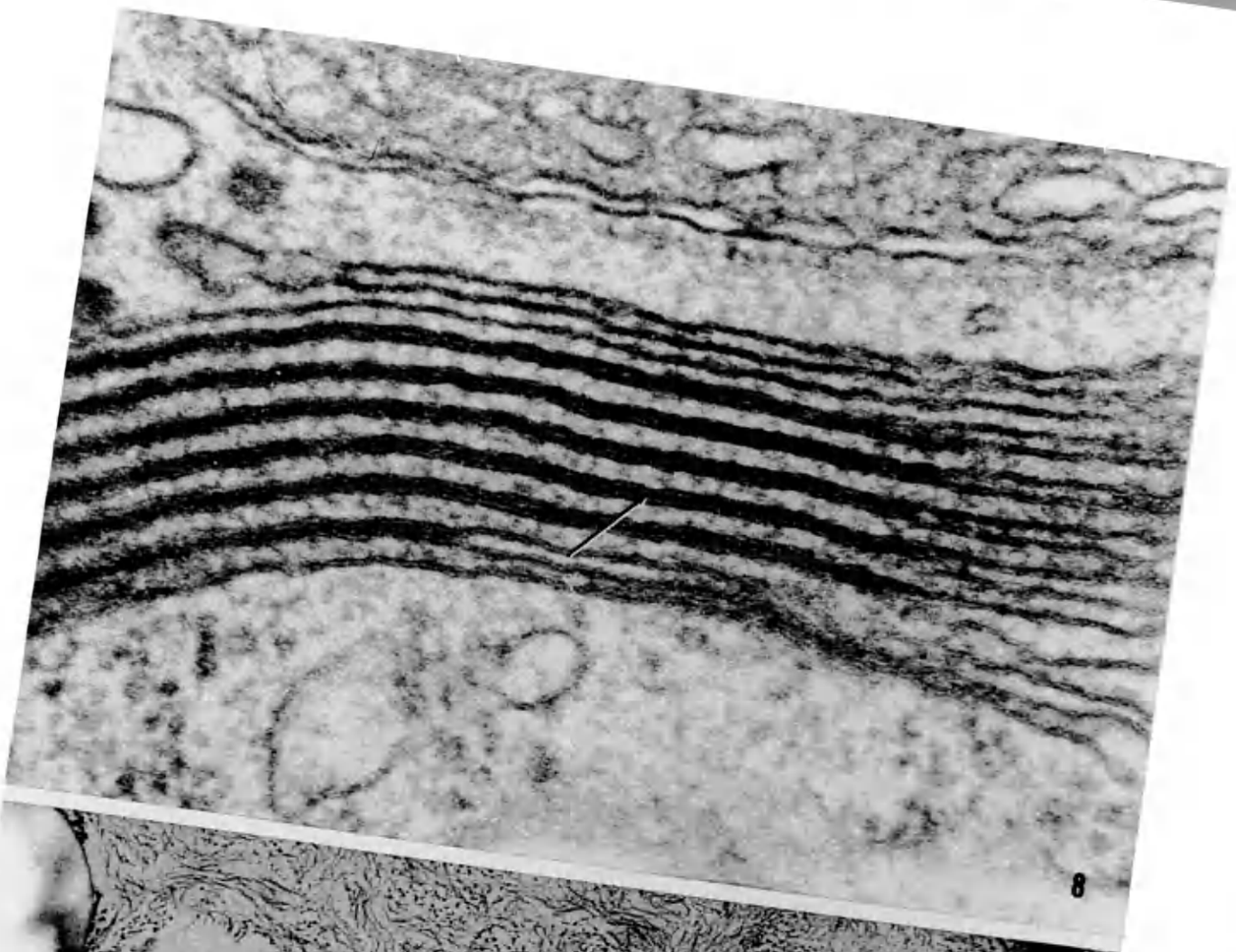

DC
NSV
V
M
ER
7

PLATE IV

Figure 8. High magnification electron micrograph giving evidence for an intercisternal substance in the region of cisternal appression. A darkly staining intercisternal substance may be visualized between two complete membranes (arrow). X168,000.

Figure 9. Secretory vesicles (V) of the type formed by the dictyosomes in stage III tetrasporangia, releasing their contents into a large fibrous vacuole (FV). X35,625.



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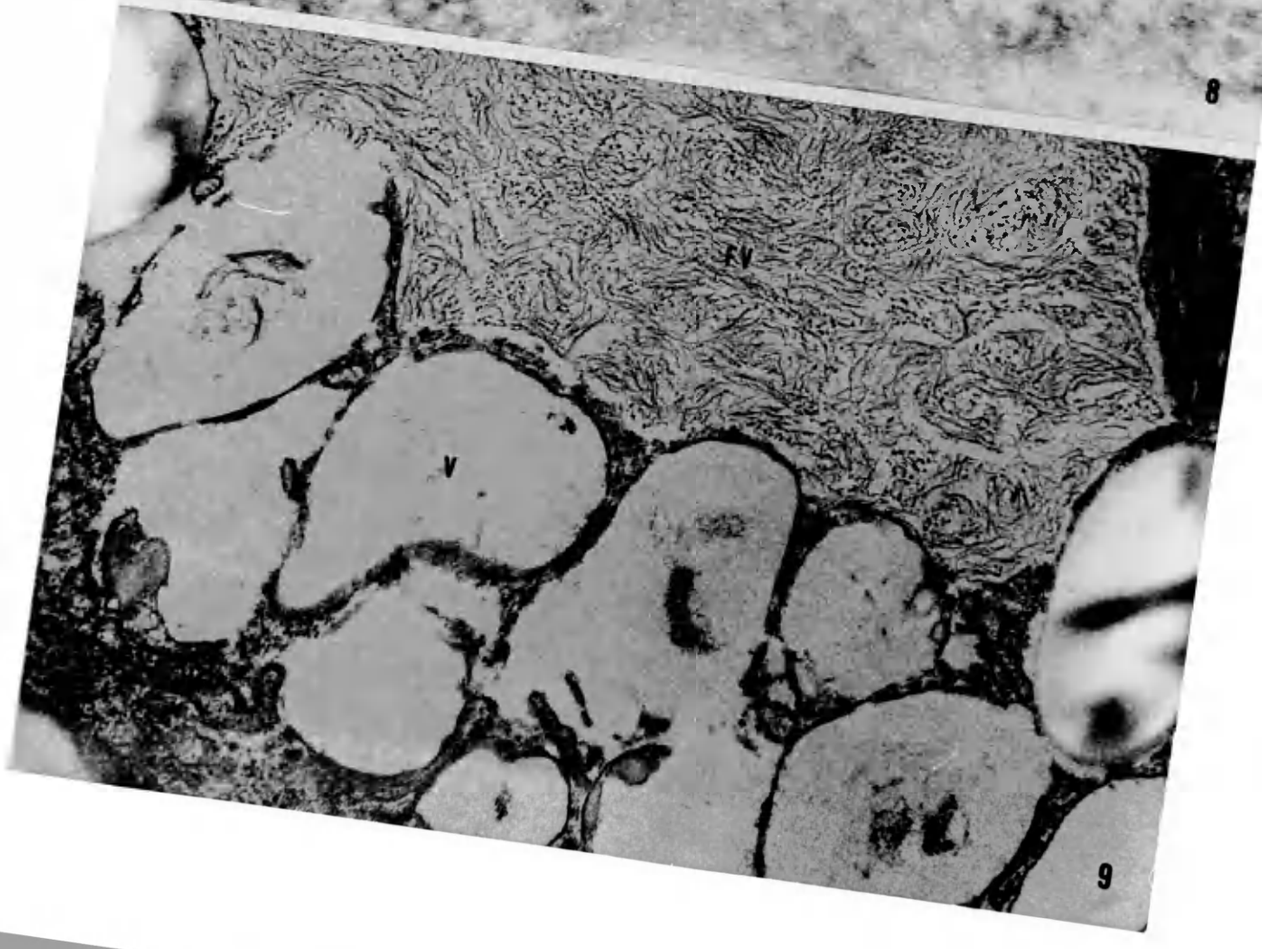

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